The Spectrum of B-Cell Non-Hodgkin Lymphomas With Dual \textit{IgH-BCL2} and \textit{BCL6} Translocations

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**Key Words:** Oncogene; BCL2; BCL6; Non-Hodgkin lymphoma; Dual translocation

**Abstract**

Dual \textit{IgH/BCL2} and \textit{BCL6} translocations are rarely observed in B-cell non-Hodgkin lymphomas (B-NHLs). We investigated the morphologic, phenotypic, and cytogenetic spectrum of B-NHL with such dual translocations.

Dual \textit{IgH/BCL2} and \textit{BCL6} translocations were detected in follicular lymphomas (FLs) and diffuse large B-cell lymphomas (DLBCLs), representing 6.1% of 132 B-NHLs in our series, including 6 (11%) of 56 FLs (grades 1, 2, and 3a) and 2 (3%) of 76 DLBCLs; 33% of FLs with dual translocations had variant morphologic features. All dual-translocation FLs were CD10+/BCL6+/BCL2+/MUM1–, and the DLBCLs demonstrated “activated” germinal center (CD10+/BCL6+/MUM1+) and non–germinal center (CD10–/BCL6+/MUM1+) phenotypes. \textit{BCL6} translocations in all cases involved nonimmunoglobulin genes/loci. Mean chromosome abnormalities in dual-translocation FLs and DLBCLs did not differ from \textit{IgH/BCL2} FLs and DLBCLs.

Detection of dual translocations predominantly in low-grade FLs suggests that \textit{BCL6} abnormalities are acquired early in the histologic evolution of a subset of \textit{IgH/BCL2}-associated FLs.

Certain recurrent or nonrandom chromosome translocations are considered characteristic of specific types of B-cell non-Hodgkin lymphomas (B-NHLs).\(^1\) A few of these translocations are also thought to be associated with distinct pathways of lymphoma development or progression.\(^2\) Follicular lymphomas (FLs) with the translocation t(14;18)(q32;q21), resulting in fusion of the immunoglobulin heavy chain (\textit{IgH}) and B-cell lymphoma 2 (\textit{BCL2}) genes, and those lacking this translocation are thought to have different biologic characteristics.\(^3-7\) Similarly, diffuse large B-cell lymphomas (DLBCLs) harboring the translocation t(14;18) or translocations or aberrations of chromosome region 3q27, which harbors the transcriptional repressor \textit{B-cell lymphoma 6} (\textit{BCL6}) gene, are associated with distinct molecular alterations or transcriptional profiles.\(^8,9\) A few studies have described B-NHL with dual \textit{IgH/BCL2} and \textit{BCL6} translocations, which were ascertained by Southern blot,\(^10,11\) G-banded karyotype,\(^12\) or FISH\(^13\) analyses or a combination of these modalities.\(^13,14\) This combination of cytogenetic aberrations, however, is uncommon, having been reported in 9% to 15% of FLs\(^15,16\) and 10% to 30% of DLBCLs.\(^16,17\)

In FL with dual \textit{IgH/BCL2} and \textit{BCL6} translocations, rearrangement and mutations of \textit{BCL6} are considered a secondary or progression-related event because the latter have been shown to arise after the \textit{IgH/BCL2} translocation by analysis of sequential samples.\(^18,19\) Previous studies have proposed a potential role of \textit{BCL6} rearrangement in disease transformation.\(^20\) In a small subset of cases, chromosome 3q27 amplifications have also been detected after transformation of FL to DLBCL by comparative genomic hybridization.\(^21\) However, the exact temporal order and time of acquisition of \textit{BCL6} abnormalities, after \textit{IgH/BCL2}
translocations and before disease progression, with regard to other cytogenetic aberrations cannot be established from these studies because other concurrent genomic and subchromosomal molecular alterations were not analyzed in most cases.

Only a limited number of studies have assessed the frequency of dual \(\text{IgH}/BCL2\) and \(BCL6\) translocations in certain types of B-NHL, all predating the current World Health Organization (WHO) classification. Moreover, there is a paucity of information regarding the morphologic features and phenotypes of B-NHL with dual translocations. Previous studies did not compare the histologic grade and architectural patterns of FLs carrying dual translocations with those that have isolated \(t(14;18)\) or \(BCL6\) rearrangements. Differences in the mean number of cytogenetic aberrations in B-NHL with dual translocations, if any, in comparison with those associated with \(t(14;18)\) have not been addressed. Lastly, the types of \(BCL6\) translocation breakpoints and \(BCL6\) partner loci in such lymphomas have not been well characterized. Thus, to address these questions, we retrospectively analyzed G-banded karyotypes of all B-NHL with \(\text{IgH}/BCL2\) translocations diagnosed at our institute during a 10-year period to detect cases with combined translocation \(t(14;18)\) and 3q27 aberrations. Fluorescence in situ hybridization (FISH) was performed to confirm \(\text{IgH}/BCL2\) and \(BCL6\) rearrangements, and a detailed histologic and phenotypic analysis was undertaken.

**Materials and Methods**

**Case Selection, Morphologic Assessment, and Clinical Information**

The results of karyotype analyses performed at our institution for all B-NHL cases submitted during a 10-year period (July 1997–June 2007) were reviewed to identify cases with the translocation \(t(14;18)(q32;q21)\). H&E-stained sections of formalin-fixed, paraffin-embedded tissue were used for morphologic evaluation. All B-NHLs were categorized and graded according to the current WHO classification system.

Staging bone marrow biopsies, if performed, were examined. Data regarding patient demographics, disease stage, and clinical outcome were obtained from the treating physicians.

**Karyotype and FISH Analysis for \(\text{IgH}/BCL2\) and \(BCL6\)**

Giemsa (G)-banded chromosome analysis was performed after overnight culture (12-15 hours) without mitogenic stimulation, using standard methods. The karyotype failure rate was less than 20% for all types of B-NHLs evaluated during the study period. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature.22 FISH analyses were performed on fixed cells from all cases using dual-color, break-apart probes for the \(BCL6\) major breakpoint region and dual-color, dual-fusion probes for \(\text{IgH}/BCL2\) (Vysis, Downers Grove, IL), using standard protocols, and 200 cells were analyzed. Fluorescence signals were captured after counterstaining with 4’,6-diamidino-2-phenylindole using the Cytovision Imaging system attached to a Nikon Eclipse 600 microscope (Applied Imaging, Santa Clara, CA). FISH analysis using dual-color, break-apart probes for the \(BCL6\) alternative breakpoint region (ABR) was performed on 1 case at Cancer Genetics (Hackensack, NJ).

**Immunohistochemical Staining and Flow Cytometry**

Immunohistochemical staining was performed with an automated staining machine (Universal staining system, DAKO, Carpinteria, CA) after antigen retrieval, as required. The EnVision Plus kit (DAKO), in conjunction with the chromogen 3,3’-diaminobenzidine, was used for detection. Primary antibodies included CD20, CD3, CD5, CD15, CD21, CD30, BCL2, BCL6, CD10, MUM1, MIB-1, and LMP1 (DAKO). All immunohistochemical stains were scored as follows: 0, fewer than 5% stained cells; 1+, 5% to 20% stained cells; 2+, 21% to 50% stained cells; and 3+, more than 50% of stained cells. In addition, the pattern of MUM1 staining (perinodular/circumferential vs diffuse) was noted.

Three- or four-color flow cytometric analysis (FACScan, Becton Dickinson, San Diego, CA) was performed according to standard methods, and data were analyzed by using CellQuest software (Becton Dickinson). The antibodies used included CD45, CD43, CD19, CD20, CD79a, CD10, κ, λ, CD2, CD3, CD4, CD5, CD7, CD8, and CD16/56 (Becton Dickinson).

**Statistical Analysis**

The Student \(t\) test was performed to analyze differences in the mean number of chromosomal abnormalities, and a \(P\) value of less than .05 was considered significant.

**Results**

**G-Banded Karyotype and FISH for \(\text{IgH}/BCL2\) and \(BCL6\)**

The translocation \(t(14;18)(q32;q21)\) was detected in 53 of 132 B-NHLs (comprising FLs and DLBCLs) with informative karyotypes diagnosed at our institute over 10 years. These B-NHLs comprised 43 (77%) of 56 FLs (39/50 nodal FLs [78%]) and 10 (13%) of 76 DLBCLs.
B-Cell Non-Hodgkin Lymphomas With Combined 3q27 Rearrangement and Translocation t(14;18): Patient Demographics, Karyotypes, and Results of Staging Bone Marrow Biopsy

Table I

<table>
<thead>
<tr>
<th>Case No./Sex/Age (y)</th>
<th>Site</th>
<th>Diagnosis</th>
<th>Karyotype</th>
<th>BM</th>
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<tbody>
<tr>
<td>1/F/88</td>
<td>LN</td>
<td>DLBCL</td>
<td>57,XX,del(1)p35i,+hr(1)(q22q32),+del(3)(q25q27)x2,+5,+del(5)[13q32],del(6)(q12),+7,+9,add(9)(q34),+11,t(14;18)(q32;q21),+del(12)(q12),+mar1-2(cap15)</td>
<td>–</td>
</tr>
<tr>
<td>4/F/33</td>
<td>FL2</td>
<td>FL2</td>
<td>46,XX,t(3;10)(q27;q11.2),t(14;18)(q32;q21),+12(1q24),+14(q21),–15,–17,–18,–mar1–4(cap3p3/66,XX)[13]</td>
<td>–</td>
</tr>
<tr>
<td>5/F/75</td>
<td>FL2</td>
<td>FL2</td>
<td>49,–5,XX,t(3;13)(q27;q22),+del(4)(q27),+del(11)(q22q21),+12(1q24),+14(q21),+15,–mar1–2,add(4)(q24)</td>
<td>ND</td>
</tr>
<tr>
<td>6/F/68</td>
<td>FL2</td>
<td>FL2</td>
<td>51,XX,–5,+del(4)(q27),+12(1q24),+14(q21),+15,–mar1–2,add(4)(q24)</td>
<td>ND</td>
</tr>
<tr>
<td>7/F/88</td>
<td>FL1</td>
<td>FL1</td>
<td>49,XX,del(1)p32,del(2)(p25),+del(1)(p32),del(2)(p25),+del(2)(p25),+del(3)(q25q27),+del(4)(q27),+12(1q24),+14(q21),+15,–mar1–2,add(4)(q24)</td>
<td>ND</td>
</tr>
<tr>
<td>8/F/69*</td>
<td>FL1</td>
<td>FL1</td>
<td>49,–5,XX,add(x)(q22),t(1;21)(p36.2;q22),+7,+del(11)(q13.1q33.1)(q24),+14(q21),+15,–mar1–2,add(4)(q24)</td>
<td>ND</td>
</tr>
</tbody>
</table>

BM, bone marrow; DLBCL, diffuse large B-cell lymphoma; FL1, FL2, FL3a, follicular lymphoma, grade 1, grade 2, and grade 3a, respectively; LN, lymph node; ND, not done.

*Rearrangement of 3q27 involving the alternative breakpoint region.

Morphologic Features and Phenotype of B-NHLs With IgH/BCL2 and BCL6 Translocations

The histologic features and phenotype of B-NHLs with dual translocations are described in Table II and illustrated in Image II. Of the 6 FL cases, 4 (cases 3, 4, 6, and 7; Table 2 and Image 2) showed a mild increase in the number of expansile follicles with variable follicular fusion (10%-30% of follicles), and 2 of these FLs (cases 4 and 6, Table 2 and Image 2) showed a pattern reminiscent of progressive transformation of germinal centers, also referred to as the “floral” variant. All except 1 FL expressed CD10, BCL6, and BCL2 (>2+ score); only a minority of cells expressed CD10 in 1 case (1+ score). Although increased MUM1 expression was not observed, 3 FLs (50%) showed MUM1+ cells in a perinodular or circumferential distribution. The FL3a also showed CD5 expression.

One of the DLBCLs had centroblastic morphology, and the other was classified as a pleomorphic DLBCL because it consisted of an admixture of centroblasts, immunoblasts, and large binucleated and multinucleated cells, including Hodgkin and Reed-Sternberg–like cells. The latter expressed CD30, but were negative for CD15 and LMP1. Both
DLBCLs expressed BCL6 and MUM1, and one also showed weak CD10 expression. Of interest, the patient with the CD10– DLBCL also had a grade 1 FL (CD10+, BCL2+) in a mesenteric lymph node, diagnosed on a biopsy performed 14 days previously (no cytogenetic analysis was performed on the latter).

Patient Demographics and Clinical Outcome of B-NHL With IgH/BCL2 and BCL6 Translocations

The patients with FLs comprised 1 man and 5 women (age range, 33-88 years; mean, 68.7 years) and those with DLBCLs comprised 1 man and 1 woman (ages 52 and 88 years).
Morphologic features and immunophenotype (CD10, BCL6, BCL2, and Ki-67 [MIB1]) of B-cell non-Hodgkin lymphoma with dual IgH/BCL2 and BCL6 translocations (cases 1 and 2, H&E stains, ×400; immunohistochemical stains, ×200; cases 3 through 8, H&E and immunohistochemical stains, ×100).
years; mean, 70 years). A staging bone marrow biopsy, performed in 2 of 6 patients with FL (1 each with FL3a and FL2) and 1 of 2 patients with DLBCL, showed marrow involvement in only the patient with FL2. Both patients with FL1 had no demonstrable lymphadenopathy on imaging studies, and the other patients had only localized disease involving the mediastinal, abdominal, or inguinal lymph nodes.

The patient with FL3a received chemotherapy (CHOP regimen), and the 2 patients with FL1 and 3 with FL2 did not receive any therapy at our institution. One of the patients with DLBCL received radiation therapy only, and the other underwent stem cell transplantation after CHOP chemotherapy. The patient with FL3a died 1 year after diagnosis, all 3 with FL2 were lost to follow-up, and 2 with FL1 were alive 1.3 and 1.5 years after diagnosis. None of the cases of FL showed evidence of transformation to DLBCL. One patient with DLBCL was lost to follow-up after 3.6 years, and the other was alive without disease 7 years after diagnosis.

**Discussion**

A variety of cytogenetic aberrations, resulting in the activation of cellular proto-oncogenes or inactivation of tumor suppressor genes, have been described in different types of B-NHLs. Although earlier studies suggested that certain genetic alterations are “primary” or initial events in FL pathogenesis, subsequent studies using G-band karyotyping, FISH, and comparative genomic hybridization analyses have shown that a few of these might be acquired secondarily, during disease progression or transformation. Previous investigators have described subsets of FLs and DLBCLs with different combinations of \(BCL2, BCL6,\) and c-MYC rearrangements, and DLBCLs with triple \(IgH/BCL2, BCL6,\) and c-MYC translocations have also been reported. In a few studies, analysis of sequential samples has demonstrated that \(BCL6\) and c-MYC translocations arise after IgH/BCL2 translocations. The presence of a \(BCL6\) translocation in a subclone of one of our FLs (case 4; Table 1) supports the secondary acquisition of \(BCL6\) in t(14;18)-associated FLs.

There is a large variation in the frequency of FLs and DLBCLs with dual \(IgH/BCL2\) and \(BCL6\) translocations in previously published studies, which reflects differences in sample size, type of B-NHL (primary vs relapsed) studied, and method of detection.

Despite previous reports of dual \(IgH/BCL2\) and \(BCL6\) translocations in FL, little is known regarding the frequency of this combination of cytogenetic abnormalities in different histologic grades of FL. Dual \(IgH/BCL2\) and \(BCL6\) translocations were detected in 8 (6.1%) of 132 primary B-NHLs diagnosed at our institution, 6 (11%) of 56 FLs and 2 (3%) of 76 DLBCLs. The majority of FLs with dual translocations in our series were low-grade FLs (84%). The frequency of B-NHL with dual \(IgH/BCL2\) and \(BCL6\) translocations in our series is slightly higher than that of Muramatsu et al., who detected 6 such cases (2.7%) in a survey of 222 B-NHLs, which included primary and relapsed cases. The lower frequency of dual translocations in our DLBCLs than reported previously most likely reflects the inclusion of only primary cases.

Previous studies have demonstrated that approximately 90% of low-grade FLs have the translocation t(14;18) (q32;q21). BCL6 translocations have also been described in minor subsets of FL that lack \(IgH/BCL2\) translocations, across all histologic grades. Recent studies have suggested that high-grade FLs, which have been divided into 2 subtypes (FL3a and FL3b), may represent a heterogeneous category. FL3b has a lower frequency of \(IgH/BCL2\) translocations, and c-MYC translocations have also been reported. In a few studies, analysis of sequential samples has demonstrated that \(BCL6\) and c-MYC translocations arise after IgH/BCL2 translocations. The presence of a BCL6 translocation in a subclone of one of our FLs (case 4; Table 1) supports the secondary acquisition of BCL6 in t(14;18)-associated FLs.

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**Table 2**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Diagnosis</th>
<th>Morphologic Features</th>
<th>CD10</th>
<th>BCL6</th>
<th>BCL2</th>
<th>MUM1</th>
<th>Ki-67 (%)</th>
<th>sIg L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DLBCL</td>
<td>Centroblastic</td>
<td>2+</td>
<td>2+</td>
<td>3+</td>
<td>3+</td>
<td>70</td>
<td>Absent</td>
</tr>
<tr>
<td>2</td>
<td>DLBCL</td>
<td>Pleomorphic†</td>
<td>0</td>
<td>2+</td>
<td>2+</td>
<td>3+</td>
<td>30</td>
<td>Failure</td>
</tr>
<tr>
<td>3</td>
<td>FL3a‡</td>
<td>Follicular fusion (20%)</td>
<td>3+</td>
<td>2+</td>
<td>3+</td>
<td>1+</td>
<td>40</td>
<td>x</td>
</tr>
<tr>
<td>4</td>
<td>FL2</td>
<td>Floral variant, follicular fusion (30%)</td>
<td>3+</td>
<td>2+</td>
<td>3+</td>
<td>0</td>
<td>10</td>
<td>x</td>
</tr>
<tr>
<td>5</td>
<td>FL2</td>
<td>Follicular fusion (&lt;5%)</td>
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<td>2+</td>
<td>3+</td>
<td>0</td>
<td>10</td>
<td>x</td>
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<tr>
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<td>3+</td>
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<td>40</td>
<td>x</td>
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<tr>
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<td>FL1</td>
<td>Follicular fusion (&lt;5%)</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
<td>0</td>
<td>5</td>
<td>x</td>
</tr>
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DLBCL, diffuse large B-cell lymphoma; FL1, FL2, FL3a, follicular lymphoma, grades 1, 2, and 3a, respectively; sIg L, surface immunoglobulin light chain expression by flow cytometry; 0, <5% labeled cells; 1+, 5%-20% labeled cells; 2+, 21%-50% labeled cells; 3+, >50% labeled cells.

† Large, pleomorphic cells express BCL2 and BCL6.

‡ Pleomorphic DLBCL showed large multuncleated cells resembling Hodgkin and Reed-Sternberg cells in addition to centroblasts and immunoblasts.

# Table 2

**B-Cell Non-Hodgkin Lymphoma With Combined 3q27 Rearrangements and Translocation t(14;18): Morphologic Features and Phenotype**

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<td>2+</td>
<td>3+</td>
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<td>x</td>
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<tr>
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<td>0</td>
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<td>1+</td>
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<td>3+</td>
<td>3+</td>
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<td>5</td>
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and higher frequencies of BCL6 aberrations compared with FL3a.7 Subdivision of FL3b into 3 cytogenetic subgroups, based on the presence or absence of IgH/BCL2 and BCL6 rearrangements, has been proposed, and the occurrence of IgH/BCL2 and BCL6 translocations is considered mutually exclusive in this subtype of FL.6 This dichotomy does not hold for low-grade FL and FL3a as exemplified by our study. Bosma-Bouwer et al39 demonstrated translocations involving the ABR of BCL6 in 6 (67%) of 9 FL3b cases, which is higher than the frequency observed in FL1/2 and FL3a (10%) and DLBCL (2.4%).40 Our findings indicate that ABR rearrangements can occasionally also be detected in conjunction with IgH/BCL2 translocations in low-grade FL.

The majority of FLs with dual translocations had morphologic features similar to those described for t(14;18)-associated FL.21 Only minimal overlap with features reported for BCL6-associated FL, especially the presence of large follicles, was seen in 4 of 6 FLs in our series.5 Two of these cases showed features of the floral variant, an uncommon morphologic variant of FL,42 that, to the best of our knowledge, has not been described in FL with dual IgH/BCL2 and BCL6 translocations. One of our DLBCL cases had pleomorphic morphology with Hodgkin-like features, similar to the DLBCL reported by Garcia et al.43

The phenotype of our FLs with dual IgH/BCL2 and BCL6 translocations (CD10+, BCL6+, BCL2+) suggests a closer relationship of these cases with conventional or t(14;18)-associated FLs.44,45 Of note, the FL3a in our series also expressed CD5, similar to FLs described by Barry et al.46 We observed minimal or no MUM1 expression in our FLs with dual translocations across all grades. The phenotype of DLBCLs after transformation of FLs with dual translocations is not known, although the non–germinal center (GC) phenotype detected in 1 DLBCL and MUM1 expression, in conjunction with CD10 and BCL6 expression by the other DLBCL in our series, suggests an “activated” GC phenotype, which is uncommon for DLBCLs associated with t(14;18).37 It must be pointed out, however, that even though the algorithm of Hans et al.47 is useful in categorizing DLBCLs into GC or non-GC types and is a good substitute for the gene expression profile–derived classification, approximately 20% of cases are misclassified by this approach. It remains unclear at present whether a subset of DLBCLs with non-GC immunophenotype or those coexpressing CD10 and MUM1 have the “genetic phenotype” of GC-type DLBCLs, ABC-type DLBCLs, or if they represent a distinct category. The impact of distinct types of BCL6 alterations48 or other genetic lesions on the immunophenotype of such DLBCLs also needs to be investigated.

The mechanistic basis for the proclivity of some t(14;18)-associated FLs to acquire secondary BCL6 rearrangements is unknown, and the clinical consequences of this phenomenon are debatable. DLBCLs with BCL6 translocations have been shown to have a better prognosis.49 However, mutations in the 5′ noncoding region of BCL6 have been proposed to play an important role in the transformation of FL to DLBCL.18 Akasaka et al20 also reported that FL acquiring BCL6 rearrangements might be at a higher risk for transformation. However, others have not observed unusual clinical features or accelerated progression of B-NHLs with combined IgH/BCL2 and BCL6 translocations.10,15,29,32,34

The predominance of low-grade FL with such cytogenetic aberrations and lack of a significant difference in the mean number of chromosomal abnormalities between FL and DLBCL with combined IgH/BCL2 and BCL6 translocations and those with IgH/BCL2 translocations (but lacking BCL6 rearrangements) in our series also argues against enhanced cytogenetic progression of FL with dual translocations. It is possible that other, as yet uncharacterized, genetic abnormalities play a more dominant role in the transformation of these FLs to DLBCL. However, the limited clinical follow-up of our patients with FL precludes predictions on the clinical outcomes of such cases.

Only limited information exists regarding BCL6 partner genes or loci in B-NHL with dual IgH/BCL2 and BCL6 translocations. A review of the literature suggests that BCL6 translocations may involve the second IgH allele,35 immunoglobulin light chain genes,32 nonimmunoglobulin genes,15,28 or, rarely, prior IgH/BCL2 translocation as part of complex rearrangements.14 All of the BCL6 partner loci identified in our series did not involve the IgH gene or immunoglobulin light chain genes. Worse survival has been reported for patients with DLBCLs that have nonimmunoglobulin BCL6 partner loci compared with those that have DLBCLs with translocations involving immunoglobulin genes.50 Both of our patients with DLBCLs had prolonged survival. An impact of nonimmunoglobulin partners of BCL6 rearrangements on the prognosis of FL, if any, is not known.

In summary, we observed a higher frequency of dual IgH/BCL2 and BCL6 translocations in low-grade FLs (FL1 and FL2), suggesting the possibility of early acquisition of BCL6 translocations during the cytogenetic progression of a subset of FLs. Our findings argue against enhanced genetic instability of FL owing to acquisition of BCL6 translocations. Overall, FL with dual IgH/BCL2 and BCL6 translocations had morphologic and phenotypic features similar to FLs that have IgH/BCL2 but lack BCL6 translocations, whereas the DLBCLs had GC and non-GC immunophenotypes.

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